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GOMPHONEMA ANGUSTIVALVA (BACILLARIOPHYTA), A NEW DIATOM RECORD FOR ROMANIA

Krisztina Buczkó[1, 2]*, Enikő Magyari[3, 4], Zoltán Szabó[2, 4] and János Korponai[2, 5]

[1]*Department of Botany, Hungarian Natural History Museum, H–1431 Budapest, Pf. 137, Hungary; *buczko.krisztina@nhmus.hu*
[2]*Centre for Ecological Research, Aquatic Ecological Institute, H–1113 Budapest, Karolina 29. Hungary*
[3]*MTA-MTM-ELTE Research Group for Palaeontology, H–1117 Budapest, Pázmány Péter stn. 1/C, Hungary*
[4]*Department of Environmental and Landscape Geography, Eötvös Loránd University, H–1117 Budapest, Pázmány Péter stn. 1/C, Hungary*
[5]*Department of Water Supply and Sewerage, Faculty of Water Science, National University of Public Service, H–6500 Baja, Bajcsy-Zsilinszky u. 12–14, Hungary*

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Abstract: Here we report and document the first Romanian occurrence of the diatom *Gomphonema angustivalva* E. Reichardt 1997 from a proglacial lake of the Carpathian Mts. Identifying this narrow-valved gomphonemoid taxon (valva width < 5 μm) requires the exploration of ultrastructure using scanning electron microscopy. We observed the species in the material collected from a lake sediment core of Lake Bâlea (Romania).

Key words: Carpathian Mts, Cryptic project, diatom taxonomy, Făgăraș Mts, *Gomphonema*, Lake Bâlea

INTRODUCTION

In the last couple of years increasing attention has been paid to the high mountain lakes as sentinels of climatic changes all over the world (Moser *et al.* 2019). Palaeolimnology – the multidisciplinary science that uses the physical, chemical, and biological information preserved in sediment profiles to reconstruct past environmental conditions in inland aquatic systems (Cohen 2003) – offers a powerful tool for detecting the status and changes of aquatic ecosystems. Lacustrine sediment can preserve remains of several biotas of the lakes; some of the buried remains are regarded as very sensitive indicators of environmental status (Catalan *et al.* 2013). Among the biotic proxies, diatoms are the most often used palaeoecological object, as they are useful bioindicators. Most of the diatoms have specific ecological preferences, exhibit a variety of life strategies,

and their short life spans enable them to fast respond to environmental changes (Rimet and Bouchez 2012).

Diatoms – (Bacillariophyta) unicellular aquatic photoautotrophs – are an abundant, diverse, widely distributed component of algal assemblages in freshwater lakes (Smol and Stoermer 2010). To make reliable environmental reconstruction accurate identification of different taxa is required. The classical, morphological taxon identifications are based on cell wall morphology (Manoylov 2014). Though the taxonomy of diatoms is well documented (e.g. Lange-Bertalot *et al.* 2017), there are several challenges in the high-quality diatom taxonomy. The diatom taxonomy is under continuous changes and developments (Stoermer 2001); applying names to different morphological variations of small and morphologically variable taxa is always challenging (Manoylov 2014).

We have been working on a diatom-based palaeoecological survey on the mountain lakes of the Carpathian Mts for almost twenty years (Buczkó 2016, Buczkó and Magyari 2007). Our previous studies discovered and documented the occurrences of several rare and new diatom taxa from the lakes situated in the Retezat and Comadul Mts (Buczkó 2016), however, these rare species often cannot get enough weight in the discussion of the quantitative reconstructions and evaluation of lake biota changes. The cryptic diversity of narrow-valved gomphonemoids was found in lake Saint Anne and discussed in Buczkó *et al.* (2018) as *Gomphonema sancti-naumii* Metzeltin et Z. Levkov in Levkov, Krstic, Metzeltin and Nakov 2007: 67, pl. 171: figs 1–15. The correct and refined identification of taxa makes a significant contribution to the knowledge of the biogeography of diatoms.

The members of the genus *Gomphonema* are the most frequently reported benthic diatoms in freshwater assemblages (Lange-Bertalot *et al.* 2017). *Gomphonema* frustules are typically heteropolar, wedge-shaped in girdle view, with a wider head pole and a narrower foot pole. Valve views are more or less clavate (club-shaped) and symmetrical to the apical axis (Levkov *et al.* 2016, Round *et al.* 1990). They inhabit a wide range of lotic and lentic habitats, from small ponds to large rivers, however, many *Gomphonema* taxa have been confused and incorrectly identified (Kociolek and Kingston 1999). Therefore it is important to gain a clearer picture of species variability of the well-known genus rare within the region. Recently more than 1,500 species names belonging to the genus *Gomphonema* are listed in the relevant databases (Guiry and Guiry 2021, Kociolek *et al.* 2021).

During the on-going palaeoecological survey of the diatom flora of Lake Bâlea we rarely found narrow (< 5 μm) diatom valves. Recognising that identifying these narrow-valved *Gomphonema* taxa is impossible without high resolution microscopy, a scanning electron microscopic (SEM) analysis was carried out.

In this paper we present a new diatom record to Lake Bâlea, a well-known mountain lake that recently became one of the most popular touristic attractions in Romania, lying very close to the Transfăgărașan Highway. Our aim is to give more solid, well-documented diatom record for the Carpathian region.

MATERIALS AND METHODS

Study site Lake Bâlea

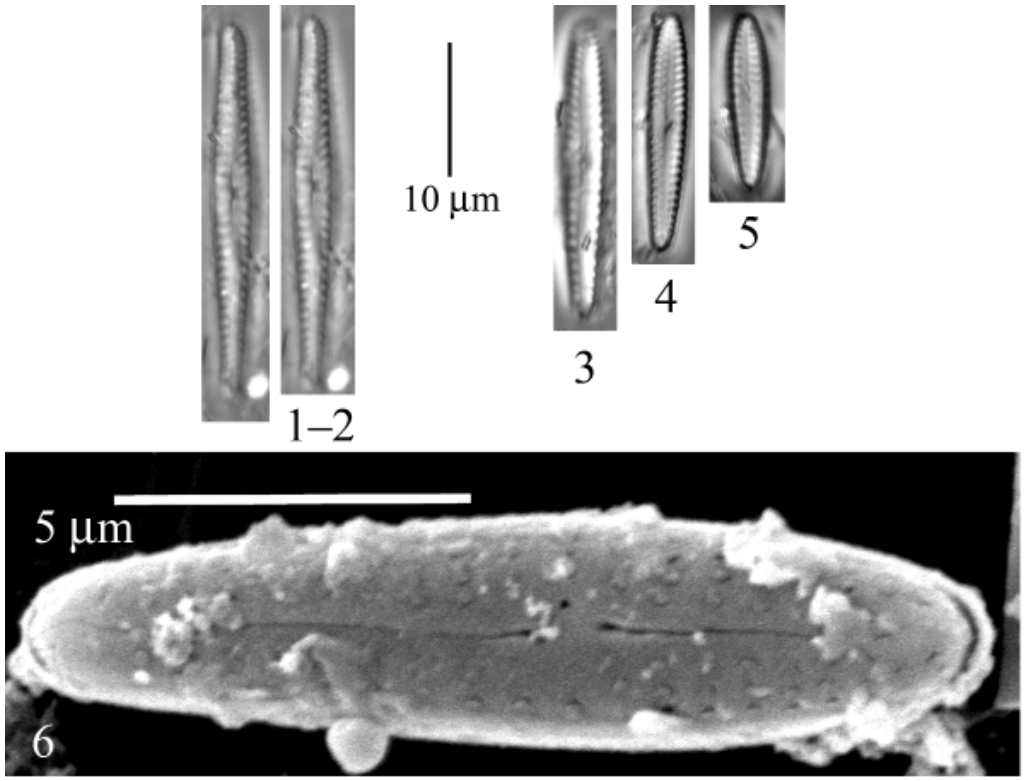
Lake Bâlea is one of the greatest proglacial lakes of the Romanian Carpathians; it is located in a homonym depression, at an altitude of 2,050 m, on the northern slope of the Făgăraș Mts, in the Southern Carpathians (Szabó *et al.* 2020). A 32-cm sediment core (Bâlea2018-01, N 45° 36' 11", E 24° 36' 55") was retrieved from the central part of Lake Bâlea (the water depth was 8 m) in July 2018. The core was sliced into 1 cm sections in the field and stored at 4 °C until further examination (Szabó *et al.* 2020). Samples for diatom analysis were prepared by standard digestion procedures (Battarbee 1986). A small portion of wet sediment was digested in hot 30% H_2O_2; then washed several times. The cleaned valves were dried on coverslip and embedded Naphrax resin. Diatom counting was conducted using a Leica DM LB2 (equipped with a 100 HCX PLAN APO objective and an Olympus SC180 digital camera) light microscope at ×1000 magnification under oil immersion and phase contrast. For SEM, cleaned samples were air-dried on an aluminium stub. Specimens were coated with gold-palladium using a XC7620 Mini Sputter Coater for 120 s at 16 mA and studied with a Hitachi S-2600N scanning electron microscope operated at 20 kV and 5–8 μm distance.

RESULTS

Nine *Gomphonema* taxa were found in the 32 cm long sediment core obtained from Lake Bâlea. The gomphonemoid taxa had low abundance in the samples; the sum of them has never reached the 4% in relative abundances of the diatom assemblages, so the occurrence of *Gomphonema* species can be regarded as sporadic during the last 500-year history of Lake Bâlea. Among them *Gomphonema pumilum* (Grunow) E. Reichardt et Lange-Bertalot (1991: 528, pl. 6: figs 4–11) and *Gomphonema minutum* (C. Agardh) C. Agardh (1831: 34) were the most frequent species, while only one valve of *Gomphonema saprophilum* (Lange-Bertalot et E. Reichardt) Abraca, R. Jahn, J. Zimmermann and Enke (2014: 11) was found in the top of the core. *Gomphonema elegantissimum* E. Reichardt et Lange-Bertalot in Hofmann et al. (2011: 302), *Gomphonema lateripunctatum* E. Reichardt et Lange-Bertalot (1991: 530, pl. 5: figs 1–19; pl. 6:

figs 1–3), *Gomphonema micropus* Kützing (1844: 84, pl. 8: fig. 12), *Gomphonema parvulum* (Kützing) Kützing (1849: 65) and *Gomphonema truncatum* Ehrenberg (1832: 88) was sporadically detected in the lake sediment.

In the sample 26–27 cm (*ca* 1680 AD) tiny, narrow valves of a gomphonemoid taxon were found. According to the identification of the valves by Lange-Bertalot *et al.* (2017), they were identified as *Gomphonema minusculum* Krasske (1932). Recently, Lange-Bertalot *et al.* (2017) is the most often used, basic guide in benthic diatom studies. All features of the two taxa fit well: the narrowly linear-lanceolate valve, widest at the centre, with obtusely rounded ends. The footpole is narrowly rounded, the striae are weakly radiate to parallel. The dimensions of all diatoms also show great agreement (the length is 14–32.7 μm, the width is 2.8–4.6 μm and the striae are 12–16 in 10 μm, usually 13–15 in 10 μm) with the description of *G. minusculum*. (Table 1). Axial area is narrowly linear, central area is transversely rectangular by bilateral regular shortening of



Figs 1–6. *Gomphonema angustivalva*. **Figs 1–5**: Valve face in light microscope. **Fig. 6**: Note the uniseriate striae and mantle. Outside view, scanning electron microscope, the same valve as in Figs 1–2 (using different focus).

the central striae. Stigmoid lies very close to the central nodule. Raphe is filiform to weakly widened, slightly undulate with narrowly spaced central pores.

Gomphonema minusculum is a widely distributed and frequent species, having wide ecological amplitude, from oligo- to eutrophic, standing freshwater habitats on carbonate to siliceous substrata; so, the bioindicator value of *G. minusculum* is rather limited.

Lange-Bertalot *et al.* (2017) mention as similar taxon *Gomphonema pseudotenellum* Lange-Bertalot in Krammer and Lange-Bertalot (1985: 51, pl. 35: figs 7–12), a rare taxon mainly known from lakes in the northern calcareous Alps and in the Balkans. It has similar dimensions and a curved raphe, but differs mainly by narrower, not protracted headpoles. During further checking the identity of these narrow-valved gomphonemoids in a recently published monograph (Levkov *et al.* 2016), we recognised that the taxonomic concept, especially regarding the dimension of the species, is basically different from the Lange-Bertalot *et al.* (2017) concept (Table 1).

The description of *Gomphonema angustivalva* E. Reichardt (1997): the valves are slightly heteropolar, linear to linear-lanceolate with narrowly rounded headpole and footpole. Valve length is 14–34 μm, width is 2.5–3.5 μm. Axial

Table 1. The dimensions of some narrow-valved (< 5 μm) *Gomphonema* taxa according to Reichardt *et al.* (1997), Levkov *et al.* (2016), and Lange-Bertalot *et al.* (2017).

Taxon	Reference	Length (μm)	Width (μm)	Number of striae in 10 μm	Characteristic features
G. minusculum	Lange-Bertalot *et al.* (2017)	14–32.7	2.8–4.6	12–16/10	
G. minusculum	Levkov *et al.* (2016)	14–34	3.5–4.5	12–15	
G. minusculum	Reichardt *et al.* (1997)	14–34	2.8–4.6	(12)–13–15–(16)	
G. pseudotenellum	Lange-Bertalot *et al.* (2017)	n.a.	2.5–4	n.a.	Acutely rounded ends
G. pseudotenellum	Levkov *et al.* (2016)	13–30	3–3.5	12–16	
G. angustivalva	Lange-Bertalot *et al.* (2017)	n.a.	2.7–3.7	n.a.	
G. angustivalva	Levkov *et al.* (2016)	14–34	2.5–3.5	15–18	
G. angustivalva	Reichardt *et al.* (1997)	11–24	2.7–3.7	15–18	
G. angustivalva in Lake Bâlea	Present study (Fig. 1)	13–28	2.6–3.1	14–16	

area is narrow, linear, central area is large. Central striae on both valve sides are distantly spaced from the other striae. One isolated pore is present in the central area. Raphe is filiform, external proximal raphe endings are tear-drop-shaped, slightly deflected towards the isolated pore (Fig. 6). Transapical striae in LM are fine, radiate in mid-valve, 15–18 in 10 μm, becoming parallel or slightly radiate towards headpole and footpole. Striae are uniseriate, composed of large c-like areolae (Fig. 6). Areolae are not distinguishable in LM, occluded by siliceous flaps.

Accepting and following the description by Reichardt (1997) and Levkov *et al.* (2016), the diatom, found in Lake Bâlea is conspecific with *G. angustivalva*.

DISCUSSION

Gomphonema angustivalva was described in 1997 by E. Reichardt during the revision of the *Gomphonema pumilum* species complex (Reichardt 1997). *G. angustivalva* was frequently observed from a variety of oligotrophic habitats (lakes, ponds, peat bogs, and small streams) in North Macedonia. According to Guiry and Guiry (2021), *G. angustivalva* has already been recorded from France, Germany, the Netherlands, and North America. Recently, Kamberović *et al.* (2019) added a new record to the occurrence of *G. angustivalva* from Bosnia and Herzegovina.

The greatest difference between *Gomphonema* species can be observed in the stria/areola structure and in the external and internal opening of the isolated pore (Levkov *et al.* 2016). Based on scanning electron microscopic features (striae uniseriate, composed of large c-like areolae), the presence of *G. angustivalva* in Lake Bâlea is the first documented occurrence of the species from Romania, and is considered to be proven. *G. angustivalva* is a diatom the identification of which is problematic; according to the most commonly used manual (Lange-Bertalot *et al.* 2017), the taxon found in Lake Bâlea is *G. minusculum*. *G. minusculum* is known as a broad-tolerant species, with low indicator value, while *G. angustivalva* is an indicator of oligotrophic waters. This makes a difference in the environmental reconstructions.

The first results on diatoms of Lake Bâlea were published by Zanoschi and Oltean (1976). In this paper the authors reported the occurrence of 110 diatom taxa, collected from 5 sampling points. Two of the five sampling localities were situated in the mire around the lake, resulting in a high number of acidophil/acidobiontic diatom taxa, belonging to the genera *Eunotia* and *Pinnularia*. Ten *Gomphonema* taxa were mentioned in this paper, but only two are shared with our

list of diatoms in Lake Bâlea, namely *G. parvulum* and *G. pumilum* (published as *Gomphonema intricatum* Kützing var. *pumilum* Grunow).

According to Caraus (2017) *Gomphonema angustivalva* in Lake Bâlea is a new record for the country.

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Summary: The correct identification of diatoms at the species level is a basic requirement in the ecological status assessment of freshwaters and in the preparation of quantitative palaeoecological reconstructions. With the improvement of microscopic technology, species concepts have also been refined. The number of taxa that can only be determined with certainty under the electron microscope is constantly increasing. The *Gomphonema minusculum* group is one of them. According to a multi-proxy analysis of Lake Bâlea, *Gomphonema* species occur rarely and with low relative abundance in the lake sediment. Based on scanning electron microscopic features (striae uniseriate, composed of large c-like areolae), the presence of *G. angustivalva* in the lake, the first documented occurrence of the species from Romania, is considered to be proven. *G. angustivalva* is a taxon the correct identification of which is problematic; according to the most commonly used manual (Lange-Bertalot *et al.* 2017), the species found in Lake Bâlea is *G. minusculum*. *G. minusculum* is known as a broad-tolerant species, and its indicator value is low, but *G. angustivalva* is an indicator of oligotrophic waters. The difference is relevant in environmental reconstructions.

Összefoglaló: A kovaalgák fajszintű határozása a felszíni vizek ökológiai állapotértékelésben és a kvantitatív paleoökológiai rekonstrukciók készítése során is alapkövetelmény. A vizsgálati eszközök fejlődésével, a mikroszkópi technológiával a fajdefiníciók is finomodnak. Folyamatosan nő az olyan taxonok száma, amelyek csak elektronmikroszkópban határozhatóak teljes biztonsággal. A *Gomphonema minusculum* csoport is ilyen. A Bâlea-tó multi-proxi elemzésének eredménye szerint a *Gomphonema* fajok ritkán és kis relatív gyakorisággal fordulnak elő a tó üledékében. Pásztázó elektronmikroszkópos bélyegek alapján (a striák c formájú areolákból állnak) bizonyítottnak tekinthető a *G. angustivalva* jelenléte a tóban, ez a faj első dokumentált előfordulási adata Romániából. A könnyen félrehatározható taxon, a jelenleg leggyakrabban használt kézikönyv alapján (Lange-Bertalot *et al.* 2017) a Bâlea-tóban talált taxon *G. minusculum*. A *G. minusculum* tágtűrésű fajként ismert, indikátor értéke csekély, viszont a *G. angustivalva* oligotróf vizek indikátora. A különbségnek a környezeti rekonstrukciókban is van jelentősége.

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